SYMPOSIUM ON PROBLEMS IN TAXONOMY[1]

CARL S. PEDERSON, *Convenor*

New York State Agricultural Experiment Station, Geneva, New York

INTRODUCTION

The session opened with a tribute to the memory of Professor Robert S. Breed, who had been associated with problems of taxonomy for many years. Professor Breed had originally organized this symposium as he had so many others in the past. Dr. C. S. Pederson, who had worked with Professor Breed for 30 years, spoke briefly of the many contributions of this great bacteriologist and stressed his leadership, friendliness, and faith in each worker in the field of microbiology. Summaries of the papers presented follow.

PART I. HOW CAN THE VARIOUS AGENCIES RELATED TO THE SOCIETY OF AMERICAN BACTERIOLOGISTS WORK TOGETHER FOR BETTER TAXONOMY AND NOMENCLATURE?

After publication of the second edition of the *Manual of Determinative Bacteriology*, Dr. David H. Bergey created an irrevocable Trust with a self-perpetuating Board of Trustees. The late Dr. R. S. Breed was editor-in-chief, and Dr. E. G. D. Murray, Dr. N. R. Smith, Dr. H. J. Conn, and Dr. R. E. Buchanan constitute the present Board. The Board, under the terms of the Trust, must use all royalties and other income for development of successive editions of the Bergey Manual.

Among the many problems facing the Board are selection of a new editor, new headquarters, and suitable facilities. The terms of Dr. Bergey's Trust permit nine members. The Council of the Society of American Bacteriologists should be requested to nominate a Society member to function as a trustee and assist in maintaining a close liaison with the Society. Likewise it would be wise for the trustees of the American Type Culture Collection also to propose one of their group as a member of the Board of the Bergey Trust.

The Bergey Manual has come to have international prestige. Perhaps some of the international microbiological societies should be invited to nominate trustees. The International Committee on Bacteriological Nomenclature of the International Association of Microbiological Societies includes representatives of more than 30 nations. The judicial committee and the editorial board of this group should work closely with the Bergey Manual Board of Trustees.

The problem of settling upon a stable taxonomy and nomenclature in bacteriology is difficult, but the several agencies enumerated have specific tasks to perform and should develop appropriate techniques of working together.

R. E. BUCHANAN

PART II. CLASSIFICATION OF THE SMALL GRAM NEGATIVE HEMOPHILIC BACTERIA ISOLATED FROM THE GENITOURINARY TRACT

a. Introduction

Classification of the gram negative bacteria found in the lower genitourinary tract of man has lacked clarity from the earliest studies until the present time. Early and differing descriptions of bacteria thought to be the cause of soft chancre were based on observations of bacteria in infected tissue and secretions and not on isolated bacteria. Unna (1895), not Ducrey, described the bacterium as a streptobacillus. Error in Bergey's Manual came from the use of an abstract for reference instead of the original paper (Ducrey,

[1] This symposium was held at the Fifty-sixth General Meeting of the Society of American Bacteriologists at Houston, Texas, May 3, 1956. Professor William G. Walter, *Montana State College, Bozeman, Montana*, served as secretary. Participants were: R. E. Buchanan, *Iowa State College, Ames, Iowa*; Margaret Pittman, *National Institutes of Health, Bethesda, Maryland*; Sidney Leopold, *Fort Detrick, Frederick, Maryland*; Charles D. Dukes, *Baylor University College of Medicine, Houston, Texas*; Reese H. Vaughn, *University of California, Davis, California*; Eric R. Brown, *University of Kansas, Lawrence, Kansas, and Fellow, National Cancer Institute*; E. Lee Treece, *University of Kansas, Lawrence, Kansas*; Kenneth L. Burdon, *Baylor University College of Medicine, Houston, Texas*; C. W. Hesseltine, *Northern Utilization Research Branch, Peoria, Illinois*; and N. M. McClung, *University of Kansas, Lawrence, Kansas*.

1895). The species name *Bacterium cancrosi* Chester, 1901, has priority over *Coccobacillus ducreyi* Neveu-Lemaire, 1921. Deacon *et al.* (1954) in a report of a gram positive phase of the soft chancre bacillus, suggested that reclassification should be considered. Severi (1953) added more confusion by proposing two new names: *Haemophilus unnae* for hemophilic bacteria that cause soft chancre, and *Streptococcus ducreyi* to include *Streptococcus giganteus* Migula, *Streptobacillus urethrae* Pfeiffer, *Haemophilus ducreyi* Neveu-Lemaire, and *Haemophilus polymorphus urethrae* Negro.

The confusion in the literature and my recent experience with cultures that were culturally different but morphologically alike and similar to *H. polymorphus urethrae*, and which I could not definitely identify using the existing descriptions, have impressed upon me the need for a comprehensive study of the bacteria of the genitourinary tract. The studies of *Haemophilus vaginalis* Gardner and Dukes (Gardner and Dukes, 1955) by Leopold and Dukes are a start.

MARGARET PITTMAN

b. Description of a Bacterium

The organism previously described (Leopold, 1953) is a pleomorphic rod, 0.5 by 0.5 to 2.0 μ, nonencapsulated, nonmotile, gram negative, and microaerophilic. Its isolation is made from a specimen streaked on Casman's agar and incubated in the presence of 10 per cent carbon dioxide for 48 hr at 37 C. The colonies are tiny, pinpoint, colorless, and surrounded by a zone of colorless hemolysis. A heavy inoculum in fluid thioglycollate medium produces "puff-ball" growths below the aerobic area. The bacterium does not reduce nitrates or produce oxidase. In the presence of cysteine trypticase agar base, acid without gas is produced from glucose, maltose and dextrin, variable and weak acidity from xylose and arabinose, and no acid from sucrose, lactose, inulin, mannitol, and glycerin.

The organism has been isolated only from the urine of men showing signs of mild to moderate prostatitis and the cervical os of women showing signs of cervicitis. When isolated it is either in pure culture or the predominating organism. Infection of small animals by the intraperitoneal route has been unsuccessful.

The morphology and growth requirements suggest a close relationship to the genus *Haemophilus*.

SIDNEY LEOPOLD

c. Growth Requirements

Small gram negative hemophilic bacilli isolated from the genitourinary tract are not readily amenable to classification by the taxonomic criteria currently accepted. These bacteria, of which *Haemophilus vaginalis* is an example, apparently possess complex growth requirements which are satisfied by blood or other body fluids, but which are in excess of the classic X and V factors. The "stimulatory" or "accessory" factors often mentioned in the literature concerning the recognized species of the genus *Haemophilus* may be determined, eventually, to constitute major growth factor status for newly recognized hemophilic bacilli in the future. Precedence for this has been established in the cocarboxylase requirement of *Haemophilus piscium*.

CHARLES D. DUKES

PART III. SOME COMMENTS ON SOURCE AND ECOLOGY

As our knowledge increases, it is becoming more and more evident that some of our former ideas of source and habitat of the bacteria are not correct.

Recent isolation of three species of *Propionibacterium* from spoiled olives is a case in point. These organisms have been of most interest to the dairy bacteriologist for years. They are common in cow dung, silage, and dairy products. A similar species, *Propionibacterium acnes*, is carried by the human. It is my belief that a statement in the generic description of the Manual would be sufficient to establish that the propionic acid bacteria are widely distributed in nature where lactates and other fermentable compounds are available.

Similar cases exist with species of *Clostridium* and *Bacillus*, not to mention several different genera of enteric bacteria. The ubiquity of these different genera makes it difficult if not impossible to be certain of the normal habitat.

REESE H. VAUGHN

PART IV. FURTHER STUDIES ON THE RELATIONSHIP OF *Bacillus cereus* VAR. *mycoides*

The Nonmotile Variant of Bacillus cereus var. mycoides

Anthrax is a disease of antiquity, the causative agent, *Bacillus anthracis*, being characterized as nonmotile, encapsulated, aerobic, sporeforming, gram positive rods, usually nonhemolytic, patho-

genic for mice and guinea pigs, slowly reducing methylene blue, producing acidity in salicin, and having a fir tree configuration in gelatin. The organisms (Ford's "anthracoides") most often confused with *B. anthracis* are characterized as being nonpathogenic, aerobic, sporeforming rods, showing marked hemolysis, and generally more active biochemically than *B. anthracis*. In previous publications (Brown and Cherry, 1955; Brown *et al.*, 1955) it was shown that a bacteriophage isolated from one strain of *B. anthracis* was capable of introducing motility to other strains of this organism, indicating that a close genetic relationship exists among certain members of the genus *Bacillus*. Recent studies utilizing over 100 strains each of *B. anthracis*, *Bacillus cereus*, and *B. cereus* var. *mycoides* have confirmed the findings of Smith *et al.* (1952), Nordberg (1951, 1953) and Seidel (1954), that there is no single criterion including pathogenicity which clearly separates members of this group of organisms, and that there seems to be a transition gradient between the "typical" anthrax bacillus and *Bacillus cereus*. Following the technique of Gordon (1940) we have been able to isolate a bacteriophage from *B. mycoides* which selectively induces both nonrhizoid, nonmotile variants of this *Bacillus* as well as nonrhizoid, motile forms. The nonmotile forms after rapid passage (3-hr) on blood-dextrose medium are pathogenic for mice, guinea pigs, and rabbits in small doses and generally exhibit characteristics associated with the "anthrax" organism. Using the technique of Thorne *et al.* (1954) it was possible after animal passage to isolate a glutamyl-polypeptide from each of these organisms which appears identical after electrophoretic, chromatographic, and Warburg and immunological studies. A method for studying immunological similarities among these organisms using the agglutination procedure with 4- to 6-hr shaken broth cultures, standardized at an optical density of 0.3 against antisera prepared from whole cell formalinized vaccines, has proved successful in our hands. Preliminary investigations indicate common antigens exist among the members of the "anthrax-cereus-mycoides" group. These studies have led us to the conclusions postulated by Smith *et al.* (1952) and accepted by many investigators in this field, that the nomenclature should be: *Bacillus cereus* var. *mycoides*, *Bacillus cereus* var. *anthracis*, and *Bacillus cereus*. It would seem that there is as much justification for designating the anthrax bacillus a pathogenic variety of *B. cereus*, as for giving varietal status to *B. mycoides* by reason of its colonial variations. The degree of pathogenicity and motility in the so-called *B. anthracis* organisms certainly varies in stock cultures and is not a reliable, stable characteristic upon which to base a species separation. Indeed, there is now considerable evidence indicating that *B. anthracis* may exist, as a result of either natural or artificial processes or by a combination of these, in any of the following forms: pathogenic, nonmotile; pathogenic, motile; nonpathogenic, nonmotile; and nonpathogenic, motile. The first of these is the typical *Bacillus anthrax* and the last is *Bacillus cereus*.

ERIC R. BROWN AND E. LEE TREECE

b. Comments

Evidence has been presented for the transformation of nonmotile anthrax bacilli to motile strains under the influence of a bacteriophage, and other studies have indicated that cultures of *Bacillus cereus* when transferred on blood medium with special frequency acquire the capacity to produce anthrax-like diseases in animals. All this would support the concept that *B. cereus* and *Bacillus anthracis* are even more closely related than has been realized, and may justify the view that the anthrax bacillus should be regarded not as a separate species, but as a variant of *B. cereus*. It seems especially important that the interrelations among the cereus-mycoides-anthracis organisms be studied by the use of strains the full laboratory history of which is known to the investigator. In my work with these organisms, I have been impressed with the clear distinction which is readily made between a virulent anthrax bacillus on the one hand, and a typical strain of *B. cereus* on the other hand. Moreover, the considerable number of attenuated strains of known anthrax bacilli studied in my laboratory have remained distinguishable from *B. cereus*, despite the fact that they did show, in varying degrees, *cereus*-like properties. Hemolytic strains of *B. cereus* have killed mice, but not with the pathological changes characteristic of anthrax. Further work along the lines opened up by Dr. Brown is clearly indicated.

KENNETH L. BURDON

PART V. COMPARATIVE TAXONOMIC STUDIES OF THE ACTINOMYCETES

a. The Genus Streptomyces

The bases which various monographers of the genus *Streptomyces* have used for the identification of species were reviewed briefly. The various characteristics such as microscopic morphology, soluble pigment, etc., which have been used as the principal means for separating species were pointed out. The treatments of Krainsky (1914), Waksman and Curtis (1916), Waksman (1919), Krassilnikov (1949), and Baldacci *et al.* (1953) were emphasized.

Practically every characteristic which can be used in differentiating *Streptomyces* species has been used by these investigators except perhaps spore markings, which recently have been beautifully illustrated by several European investigators (Baldacci and Grein, 1955; Kuster, 1953), using the election microscope.

C. W. HESSELTINE

b. The Genus Nocardia

At present the genus *Nocardia* includes microorganisms which are aerobic, partially acid-fast or non-acid-fast, and form a mycelium, which sooner or later fragments into bacillary and/or coccoid cells. The morphology of young colonies seems to offer a good method for deciding whether or not a particular microorganism should be classified in the genus. From serial studies of single cells as they develop into young colonies the genus can be divided into three groups.

Group I includes members of the genus which have sparsely branched, ephemeral mycelia, and in which fragmentation, principally of type 1, begins early (McClung, 1949). The colonial texture of these organisms is always soft, and may be mucoid. There are microorganisms which on this basis alone might be assigned to the genera *Mycobacterium*, *Corynebacterium*, or *Nocardia* with equal justification.

Group II nocardias are "typical" in that there is an extensive mycelium developed with many branches, fragmentation is delayed and occurs most often in the manner designated as type 2 (McClung, 1949). The colonial texture is usually pasty.

Group III contains organisms which have a more extensive mycelium in which fragmentation is delayed if it occurs at all. The colonial texture may be crusty, cartilaginous, or leathery. Aerial hyphae are often produced by these organisms. This group contains the pathogenic *Nocardia asteroides*, certain isolates of which, however, may be morphologically Group II (McClung, 1954*a*). There is a problem of deciding which members of this group actually are asporogenous streptomycetes. Studies on carbon utilization of the two genera may, however, be helpful. If we can compare the results of Pridham, Hall, and Shekleton as reported by Gottlieb (1953) with those of the author (McClung, 1954*b*), the genera *Nocardia* and *Streptomyces* differ significantly in their carbon compound utilization.

It has been found that beta-glucosidase is more common in *Streptomyces* sp. than in *Nocardia* sp. These results encourage one to hope that further physiological studies will reveal other differences between Group III nocardias and asporogenous streptomycetes.

N. M. McCLUNG

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